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EXPERIMENTAL DESIGN FOR THE USE OF VIRUS
AGAINST TUSsock MoTH IN WHITE FIR
PILOT CONTROL TEST
WHEELER PEAK AREA, HUMBOLDT NATIONAL FOREST
1960

By

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Entomologists

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INTRODUCTION

An epidemic infestation of tussock moth, covering about 5,000 acres of white fir was discovered in November 1959 on the east side of the Snake range near Wheeler Peak on the Humboldt National Forest, Nevada. The insect has been active for approximately 5 years and extensive tree mortality has occurred in the timber type at the higher elevations. The infestation is now moving down into the stands of better vigor, resulting in an average defoliation of 90 percent of the current growth.

The 1959 biological evaluation was made at a time when the insect was in the egg stage; consequently, parasites and predators were not evident. However, a few dead larvae were observed that could possibly have been affected by a virus. Experience with other tussock moth infestations has shown strong virus tendencies to exist within such populations. However, widespread tree killing may occur before the native virus destroys the tussock moth population. The Wheeler Peak infestation has reached the point where the damage level necessitates control action if the white fir stands in this area are to survive.

Recent work indicates that a generic-specific virus has good possibilities as a method of controlling tussock moth outbreaks. The Wheeler Peak area is an ecological timbered island isolated by desert from other timbered areas, thus it should provide an ideal test area for aerial application and establishment of the virus disease.

PAST WORK

The virus to be used was first discovered as the controlling agent of a tussock moth infestation on Douglas-fir in Owyhee County, Idaho. Extensive laboratory tests provided information on dosage required to obtain the desired mortality. Field tests in 1959 resulted in virus establishment within a tussock moth infestation on bitterbrush near Carson City, Nevada. The higher dosages up to 100,000 polyhedral bodies per acre produced up to 90 percent mortality. Massey used this virus in

a field test on tussock moth affecting white fir in New Mexico and obtained between 30 and 50 percent mortality. Laboratory tests here using Idaho virus against New Mexico tussock moth resulted in up to 86 percent reduction in the test larval population.

At this time laboratory tests are being conducted to determine the virus concentration to be used on the pilot control test. These tests are being conducted with the Idaho virus against tussock moth larvae from Wheeler Peak at rates comparable to 25×10^6 , 50×10^6 , 100×10^6 polyhedra per acre.

25, 50, & 100 million

OBJECTIVES

The objective of a pilot test is to determine if the tussock moth virus can be established by means of aerial application, and to evaluate the results of mortality caused by each dosage. The greatest reduction may take place in 1960 due to immediate establishment of the introduced virus alone or by the introduced virus aiding a natural virus by supplying added effectiveness.

On the other hand, only an establishment of virus may be obtained in 1960 which would result in a greater controlling influence in 1961. Consensus of workers in biological control is that not more than 50 percent reduction during the first year should be anticipated due to the virus. Other factors such as parasites may cause additional reduction. In any case, if the virus can be established there should be no need to respray this particular area to control tussock moth in the near future.

METHODS

This section will cover three main phases: I. Application, II. Quality control in sampling, and III, Analysis of data.

I. Application of the virus

A. Plots

1. Number and size--four plots as near equal size as possible (approximately 1,000 acres each) will be sprayed with virus solution and two check plots (approximately 500 acres each) will be sprayed with the carrier solution only.

B. Treatment

1. Virus solutions

The concentration of virus will be as follows unless the laboratory tests strongly suggest an increased or decreased amount:

- a. Two of the spray plots will be treated with the formulation containing 100×10^6 polyhedra per gallon.
 - b. Two of the spray plots will be treated with the formulation containing 100×10^7 polyhedra per gallon.
 - c. The two check plots will be sprayed with the carrier formulation without virus.
2. Rate of delivery will be 1 gallon per acre.
3. Formulation
- a. The basic formulation will be .1 gallon of carrier (nonchlorinated water) .01 gallon of a fluorescent tracer (Leucophor C).
 - b. The virus concentrations will be added to the above formulation at the rate of:
 - (1) 1 ml. of 100×10^6 polyhedra/ml./gallon
 - (2) 1 ml. of 100×10^7 polyhedra/ml./gallon
 - (3) No virus added for check spray
4. Timing--Since the virus must be ingested and the "incubation" period approaches the larval life span, spraying should be done early in the larval life in order to be effective. Spraying any time after egg hatch is approximately 50 percent complete, and ending before approximately 50 percent of the larvae are in the instar IV is planned.
5. Airplanes
- a. Class--Due to the small size of the test plots the airplanes used will be of the Stearman to Ford class.
 - b. Height of spraying--Due to evaporation rate of water and loss of the polyhedra, the height of spraying should be the minimum that safety will allow (approximately 100 feet above the tree tops).
 - c. Flight speed--Again evaporation is the controlling factor, hence spraying speed should average 80-100 m.p.h.

II. Quality control of sampling

A. Population sampling

1. Cages--Immediately after spraying a sleeve cage (18 inches long, 9 inches in diameter) will be placed over a branch of each of 10 white fir trees per plot. These cages will cover the terminal end of one branch per tree. Prior to the placement of the cages, the larval population per caged portion of the branch will be adjusted (added to or depleted) so that all cages will contain equal populations.
2. Larval sampling--Checks of the cages will be made twice a week for the first two weeks and then 3 times a week until pupation. The number of dead and surviving larvae will be recorded for each cage. Periodic checks will continue until moth emergence is completed.
3. Collecting--Dead larvae will be collected and placed individually in vials in order to determine cause of death. Vials will be sterilized, dry, and used only once on this project. No preservatives will be used in the collections.

B. Insecticide sampling

1. Spray deposit plates--3" x 3" smoked glass plates are recommended for determining spray coverage. The insecticide is of water base, thus the standard oil-sensitive dye cards are not usable.
2. Fluorescent tracer--Leucophor C fluoresces under ultraviolet light, thus by use of a "light" or "blackbox", spray droplets can be counted on the smoked glass plates.
3. Sampling spray coverage--Spray deposit plates will be distributed at equidistant intervals along a line crossing the spray pattern at right angles. The distance between plates should approximate 5 chains and, if possible, a check line established at each end of the spray block. In addition, two plates should be placed within close proximity of each sleeve cage.

C. Analysis of data

1. Abbott's formula will be used to obtain early estimates of percent mortality due to the virus. This is:

$$\frac{x-y}{x} \times 100 = \text{percent control due to treatment}$$

Let: x = percent living in check plot
 y = percent living in treated plot
 $x-y$ = percent killed by treatment

2. Analysis of variance will be used to test for mortality differences between virus concentrations. The analysis of variance form is:

<u>Source</u>	<u>Degree of freedom</u>
Between virus	1
Within virus	38
Total	39

3. Probit analysis will be used to determine the relative potency of the virus preparations as compared to the check areas.

MATERIALS

This section will attempt to bring together: I. Estimates of manpower, and II, the major materials needed for the technical portion of the project.

I. Manpower requirements

<u>Position</u>	<u>Number</u>	<u>Int. Station</u>	<u>N.F.A.</u>
Entomologists	2	40	
Biologists	1	30	
Checkers	2		80

II. Materials needed (based on 5,000 acres)

<u>Item</u>	<u>Quantity</u>
Insecticide	
Nonchlorinated water	4,500 gal.
Evaporation inhibitor (light corn syrup)	500 gal.
Virus concentration	
100 x 10 ⁶ /ml.	2,000 ml.
100 x 10 ⁷ /ml.	2,000 ml.
Leucophor C ^{1/}	50 gal.

^{1/} A product of Sandoz Inc., Chemical Div., 61-63 Van Dorn St., New York 13, N. Y.

<u>Item</u>	<u>Quantity</u>
Sampling	
Sleeve cages	
Acetate sheets .020 ga. (18" x 30")	60 sheets
Plastic screen - 36" wide	120 yards
Ultraviolet light or "blackbox"	4
3" x 3" smoke glass plates	200
Vial shell 9 x 30 mm., 1/4 dram	7 gross
Corks, 0	7 gross
Gum labels, 1" sq.	7 gross
Dissecting needle	7
Forceps	7
Marking twine	12 cones
Tree tags or	100 tags
Plastic ribbon	6 rolls
Laboratory	
Laboratory microscope (430X)	1 (on hand)
Haemacytometer kit	1 (on hand)
Tally counter	1 (on hand)
Chlorox	1 gallon
Distilled water	10 gallon
General	
Forms, sampling	100 copies (on hand)
Guidelines	1/worker (on hand)
Pencils	2/worker
Notebook, pocket	1/worker
Vehicles:	
Entomologists	2 (on hand INT)
Biologist	2 (on hand INT)
Checkers	2 N.F.A.
Horses	6
Radio (handy talkies or mobile)	1/vehicle (on hand)
Altimeter	5 (on hand)
Maps - type	1 each
Aerial photos	2 sets

Costs - D. F. I. R. \$2,500

\$2,000 F.Y. 1960
500 F.Y. 1961

APPENDIX

Formulation for 100 gallons of insecticide

90 gallons chlorinated water
10 gallons evaporation inhibitor
1 ml. stock virus concentration
1 gal. Leucophor C